Phagocyte function and cytokine production in community acquired pneumonia


Abstract

Background – It is possible that many deaths from pneumonia may involve the generation of inflammatory mediators and tissue damage by activated phagocytes. To test this hypothesis phagocyte function, plasma levels of interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), and soluble interleukin 2 receptor (IL-2R), disease severity, and outcome have been examined in 46 patients with community acquired pneumonia.

Methods – Polymorphonuclear leucocyte (PMNL) and monocyte function were measured daily by chemiluminescence in these patients during the first week of admission, and cytokine levels were subsequently determined by ELISA. A series of 61 healthy individuals were used as a control group for the chemiluminescence results.

Results – There was evidence of phagocyte, particularly PMNL, activation on admission in 76% of the patients. Most patients (86%) also had raised IL-2R levels on admission. IL-6 and unbound TNFα were present in 23% and 41% of patients at varying times during the course of the disease. There was little correlation between measurements of cytokine or phagocyte levels and outcome or indicators of disease severity, although this may be because of the small number of patients included in this preliminary study.

Conclusions – These results are consistent with the hypothesis that activated phagocyte function and raised levels of circulating cytokines may contribute to the pathogenesis of community acquired pneumonia. There are striking similarities in this respect between pneumonia, adult respiratory distress syndrome, and sepsis.


Community acquired pneumonia is a considerable problem in terms of its morbidity, mortality and use of hospital resources. It causes 10 times as many deaths in the UK as all other infectious diseases together, and the subgroup of patients who have pneumococcal bacteraemia have an even greater mortality which reaches 30% in some studies. In 1964 Austrian and Gold showed that the mortality curves of patients with pneumococcal pneumonia who did not receive antibiotics and those treated with penicillin diverged after four days. Intravenous penicillin eradicates viable pneumococci from sputum in a few hours, so it seems evident that the invading bacteria trigger a pathological process which leads inexorably to multiple organ failure and death.

The involvement of phagocytes in pneumonia is well known. Infiltration of affected lung parenchyma by neutrophils occurs within a short time in response to infection, and is followed by an influx of monocytes. Killing of bacteria by antibiotic treatment is associated with resolution of this inflammatory response. However, some patients in whom resolution does not occur show pathological features which are similar to those seen in the adult respiratory distress syndrome (ARDS). Multiple organ failure is often present in patients with ARDS, as it is in patients with pneumonia who do not respond to treatment.

There is compelling evidence that mediator release from phagocytes is central to the endothelial cell damage which seems to be the hallmark of ARDS. The role of cytokines and, in particular, tumour necrosis factor alpha (TNFα) in this process has been closely investigated in ARDS and septic shock. Since phagocytes are potent producers of TNFα, we have studied phagocyte function and circulating cytokine levels in patients with pneumonia to determine whether phagocyte function and cytokine levels are abnormal in these patients. We have recently reported preliminary results from this study, which show that many of the patients included in our study had circulating TNFα at some point during their stay in hospital.

Methods

Patients

Forty two consecutive admissions to King’s Cross Hospital, Dundee with community acquired pneumonia were included in the study. Pneumonia was defined as the presence of new shadowing on a chest radiograph in
conjunction with an appropriate clinical history and examination for which no other cause was found. Informed consent was obtained from all patients and the study was approved by the Tayside Ethical Committee. The average age of the patients was 60 years with a range of 13–87 years; 30 were men. Twelve patients had evidence of chronic obstructive airways disease and some form of pre-existing lung disease was present in a total of 16 patients. Of the 42 patients included in this sample, eight had pneumococcal infection proven by culture (five blood, three sputum), one had Klebsiella (sputum), one had Staphylococcus aureus, one had influenza B, and two had mycoplasma infection proven by serological testing. The aetiological agent was not established in 30 patients due, in part, to the loss of urine samples taken for assay of pneumococcal antigen. Six patients died. The stay in hospital of those who survived varied from 1–42 days (median 11 days), excluding one patient with social problems which prevented his discharge. Thirteen patients had received treatment from their general practitioner before admission. Consent was obtained from each patient or their relatives and 20 ml blood was taken daily starting on the morning after admission; 10 ml was taken into lithium heparin anticoagulant tubes for phagocyte assays, 5 ml into EDTA tubes, and 5 ml into a plain glass tube for serum collection. A further blood sample was taken from each survivor eight weeks after discharge for both phagocyte and cytokine assays. Clinical and other parameters collected for each patient included chest radiographic appearances, temperature, respiratory rate, presence or absence of confusion, blood pressure, arterial blood gases (if appropriate), white cell count, platelet count, lymphocyte subsets, complement level, renal and liver function.

**HEALTHY CONTROLS**

Sixty ml heparinised venous blood samples were obtained from 61 healthy volunteers aged 20–60 years (median 36 years; male:female ratio 1:0.92), the majority of whom were staff members of a local agricultural research institute. Since the results of phagocyte function tests might be influenced by intercurrent viral infection or atopy, each subject was carefully questioned about their past and present medical history, drug treatment, atopy, and general health. All subjects were asked to report upper respiratory tract infections and other illnesses occurring in the week after blood donation. Those reporting such infections or illnesses were excluded from the control data set.

**POLYMORPHONUCLEAR LEUCOCYTE (PMNL) AND MONOCYTE PREPARATION**

PMNLs and monocytes were obtained by density centrifugation and their function assessed by lucigenin-enhanced chemiluminescence assay as previously described, with the following modifications.12–14 The cells were separated by layering anticoagulated venous blood over Lymphoprep (Nycomed UK Ltd, Birmingham, UK) and dense ficoll. The dense ficoll was prepared by mixing 21 g Ficoll 400 (Pharmacia Ltd, Milton Keynes, UK) dissolved in 150 ml sterile distilled water with 53 g sodium diatrizoate (Sigma Chemical Co, Poole, UK) dissolved in 80 ml sterile distilled water, and adjusting the specific gravity to 1.19. The chemiluminescence response to phorbol myristate acetate (PMA), serum opsonised zymosan (SOZ), zymosan and buffer (HBSS control) was measured in a Dynatech ML1000 luminometer using white plastic microtitre plates (Dynatech).

**CYTOKINE ASSAYS**

Assays for IL-6 and TNFα were performed by enzyme linked immunosorbent assay (ELISA). IL-6 levels were measured using a commercially available kit (Medgenix Diagnostics, Fleurus, Belgium), while free (non-bound) TNFα was measured by an ELISA system developed by Engelberts et al.15 Soluble interleukin 2 receptor (IL-2R) in plasma was measured by ELISA (Cellfree, T Cell Sciences, Cambridge, USA). Normal range data provided with both assays was used to determine whether results from patients with pneumonia were normal or abnormal.

| Total chemiluminescence (in RLU) produced by polymorphonuclear leucocytes (PMNLs) and monocytes from patients with pneumonia in response to no stimulant (HBSS), phorbol myristate acetate (PMA), serum opsonised zymosan (SOZ), and zymosan (ZYM). The first sample and average response during admission are shown (median and 95th percentiles), together with the control series data (median and 95th percentiles). |
|---|---|---|---|
| **HBSS** | **PMA** | **SOZ** | **ZYM** |
| **PMNLs:** | | | |
| 1st sample | 2.3 | 31.5 | 30.0 | 5.9 |
| Average | 0.2-43.7 | 43.3-191.5 | 34-106.4 | 0.4-105.0 |
| Admission | 8.0-15.0 | 0.5-29.0 | 46.5 | 21.2 |
| Control series | 0.5 | 24.6 | 14.5 | 2.3 |
| 0-2-18 | 13.0-57.0 | 6.1-39.8 | 0.9-6.3 |
| **Monocytes:** | | | |
| 1st sample | 3.7 | 18.0 | 32.4 | 5.9 |
| Average | 0.4-39.1 | 2.4-220.5 | 47.1-184.5 | 0.7-64.7 |
| Admission | 9.6 | 48.1 | 52.6 | 19.8 |
| Control series | 6.7-20.3 | 37.0-84.1 | 42.4-92.9 | 12.1-39.3 |
| 0.9 | 18.4 | 28.4 | 5.1 |
| 0.3-1.6 | 7.5-42.4 | 6.7-49.4 | 1.4-15.2 |

**DATA ANALYSIS**

The results were analysed as profiles for individual patients and as grouped data for comparison of patients with control subjects. Linear regression analysis was used to examine the relation between the parameters measured, and the results were compared with a healthy control population using the Kolmogorov-Smirnov (KS) test. The 95th percentiles describing the control population chemiluminescence data were used to determine the number of patients with chemiluminescence responses above the normal range. Differences between data grouped according to prognosis and outcome were compared by the Wilcoxon rank sum test.

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**Total chemiluminescence (in RLU) produced by polymorphonuclear leucocytes (PMNLs) and monocytes from patients with pneumonia in response to no stimulant (HBSS), phorbol myristate acetate (PMA), serum opsonised zymosan (SOZ), and zymosan (ZYM). The first sample and average response during admission are shown (median and 95th percentiles), together with the control series data (median and 95th percentiles).**
Results

PHAGOCYTE CHEMILUMINESCENCE

The chemiluminescence results are summarised in the table. On admission the PMNLs from 32 patients (76%) showed high background chemiluminescence production over the duration of the assay without added stimulant in comparison with a group of 61 healthy controls tested on a single occasion (KS test, p < 0.0001). PMNLs from 30 (71%) of the patients also produced a higher chemiluminescence response to stimulation by unopsonised zymosan than controls (KS test, p < 0.0001). The patients who had raised PMNL chemiluminescence responses to PMA and SOZ in comparison with controls, although this was less significant than the non-specific responses noted above (KS test, p < 0.01 and p < 0.002 respectively). Although 20 patients (48%) showed increased PMNL responses to PMA on admission, there were also six patients (14%) whose responses were reduced in comparison with the normal range. In those patients who had not received treatment before admission (fig 1) the PMNL chemiluminescence response to PMA conformed to two patterns over the first week: (1) a high initial value followed by a slow decline to normal levels (6/20); and (2) a low or normal initial reading followed by a gradual rise (14/20).

Monocyte chemiluminescence responses were also raised compared with healthy controls, with high initial responses to PMA in 20 patients (48%, KS test, p < 0.05), to SOZ in 15 patients (36%, NS), and to unopsonised zymosan in 14 patients (33%, NS). However, as with PMNLs, the striking feature was the high unstimulated monocyte production of chemiluminescence by 30 patients in comparison with healthy controls (71%, KS test, p < 0.0001). The monocyte chemiluminescence responses on admission showed good linear correlation with the initial PMNL chemiluminescence responses for both PMA (r = 0.778, p < 0.0001) and SOZ (r = 0.704, p < 0.0001).

CYTOKINES

As we have previously reported16 16 of 39 patients (41%) in whom TNFα measurements were obtained had free TNFα in their plasma at some point during their illness, nine on admission and seven subsequently. Circulating TNFα was present in two of the six patients who died. TNFα levels ranged from 10 to 354 pg/ml (median 76 pg/ml). The number of days on which TNFα was found in the plasma varied from one to four. All seven patients in whom circulating TNFα was found after admission had not received antibiotics before admission.

Interleukin 6 was present in the plasma in only eight of 34 patients, five of whom also showed TNFα at some stage during their illness. It preceded the appearance of TNFα in all but one of these five cases. IL-2R was raised on admission in 32 of 37 patients (86%, median 1286 U/ml, range 483–4899 U/ml), but only five patients showed a rising IL-2R level during their hospital admission (fig 2).

RELATION BETWEEN INDIVIDUAL IMMUNOLOGICAL PARAMETERS

The relation between the laboratory measurements was difficult to assess since the timing and speed of pathogenetic events in the patients was unknown. In patients who were not treated with an antibiotic before admission to hospital, examination of the phagocyte results showed that these also tended to rise after treatment, and the IL-6 level rose before TNFα in three out of four patients in whom both cytokines were measured (fig 3).

RELATION BETWEEN IMMUNOLOGICAL AND CLINICAL PARAMETERS

The British Thoracic Society (BTS) identified patients with a poor prognosis as those having
Phagocytes in patients with community acquired pneumonia are activated in comparison with healthy control subjects, as they are in ARDS and other inflammatory disease states. Both PMNLs and monocytes produce more reactive oxygen species without stimulation than controls. These cells are also primed to produce larger responses than controls when stimulated with PMA or opsonised particles. Their enhanced ability to respond to non-opsonised zymosan may reflect changes in the cell membrane which promote non-specific phagocytosis.

In agreement with a recent report, most patients had high levels of soluble IL-2R in their plasma on admission (fig 1), suggesting that they had substantial lymphocyte-mediated immune responses. Since it is usually assumed that pneumonia reflects a non-specific acute inflammatory response to infection, this finding emphasises the fact that potentially pathogen-specific immune responses are involved at an early stage.

One of the problems of studying pneumonia is that of knowing, with any degree of accuracy, when the infection started or the rate of progression of the illness. However, those patients who reached hospital without having received antibiotic treatment are a useful subgroup since they allow the effects of current treatment strategies on the host response to be evaluated. Many of those patients who were not treated before admission had low or normal phagocyte chemiluminescence on admission. As phagocyte chemiluminescence rises after admission and antibiotic treatment in this group of patients, it seems likely that it is not due to prior stimulation and phagocyte "fatigue." It is possible, however, that either the more activated cells in non-treated patients have been sequestered in the lung and are therefore not present in the peripheral blood, or that the phagocytes are being primed as a result of antibiotic treatment. These two explanations are not mutually exclusive, since a small proportion of activated phagocytes would not be detected by the phagocyte chemiluminescence method used, which averages the results from 25,000 cells in each well of the microtitre plate.

Our original hypothesis was based on the similarity between fatal pneumonia and ARDS, in which TNFα and phagocyte activation have been shown to have a key role, probably through their interaction with endothelial cells. A high proportion of patients with pneumonia have circulating unbound TNFα at some stage in their illness and some have IL-6, usually at an early stage in their admission. In septic shock IL-6 levels are usually high in the first 24 hours and then decline quickly.

Since both TNFα and IL-6 may be released in large quantities from macrophages, including lung macrophages, the relation between the release of these cytokines and alterations in phagocyte function is of particular interest. One would expect measurements of phagocyte function to rise at the same time as TNFα release, and this does occur (fig 2). It also appears that IL-6 release precedes evidence of systemic phagocyte priming.

Antibiotic treatment may have major effects on these mechanisms, since it is followed by an increase in phagocyte function and the appearance of circulating TNFα in the peripheral blood of at least some patients with pneumonia. This agrees with the old clinical observation that untreated lobar (pneumococcal)
pneumonia resolved by crisis with high fever, in which endogenous pyrogens such as TNFs, IL-6, and IL-1 are known to play a major part. TNFs, lipopolysaccharide, IL-1 and other cytokines are known to mediate the expression of signals on the surface of the endothelial cell which promote PMNL adhesion. Thus our results are in agreement with the hypothesis that similar mechanisms may indeed operate in sepsis and pneumonia. Since IL-6 plays a major part in the acute phase response, its appearance in the plasma of patients with pneumonia may simply reflect early inflammatory events within the lung following the release of antigen from bacteria killed by anti-biotic treatment within the first few hours of admission.

Too few deaths occurred during this study to make any firm statements about the clinical relevance of the changes we have observed. There was also little correlation between the immunological parameters measured and pro-gnostic or other clinical parameters. However, it would be premature to conclude that they have no relevance, and the six deaths (14%) in this series of patients illustrate graphically that the pathogenesis of pneumonia requires further study.

In view of the increasing evidence that TNFs release and endothelial damage by adherent phagocytes leads to the development of ARDS, it seems likely that our inability to show clinical relevance simply reflects the complex situation present in patients with pneumonia. Studies of patients with ARDS have also shown poor correlation between TNF levels or phagocyte function and outcome. It is becoming clear, however, that the timing of events is critical, and that mechanisms which downregulate inflammatory mechanisms and protect the endothelium may have as important a role as those which mediate damage. If this is the case, then it will prove difficult to predict which patients are at risk. Nevertheless, alteration of treatment regimens in community acquired pneumonia to avoid potentially injurious mediator release and phagocyte activation may be possible.

17 Ross GD, Cain JA, Lachmann PJ. Membrane complement receptor type three (CR3) has lectin-like properties analogous to bovine conglutinin and functions as receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. J Immunol 1985;134:3307-15.
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